ORIGINAL ARTICLE

Interpretive criteria of ceftibuten disk diffusion susceptibility tests according to the DIN 58 940 method

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Objective This study aimed to establish interpretive criteria for agar diffusion tests with ceftibuten disks according to DIN standards.

Methods Minimal inhibitory concentrations (MICs) and inhibition zones produced by ceftibuten in the disk diffusion test were determined for 275 recent bacterial isolates, including 11 species with 25 strains each. Regression analysis was performed for two disk loads (10 µg and 30 µg).

Results Correlation of MICs and zone diameters was good, with correlation coefficients of $r = -0.97$ for both tested disk loads. Evaluation of the calculated zone size criteria for all species showed no very major discrepancies or no major discrepancies. The 30-µg disks, however, produced unacceptably large inhibition zones for very susceptible strains, so that usage of 10-µg disks must be recommended when testing according to DIN standards.

Conclusion Based on the MIC breakpoints recommended by the DIN (≥8 mg/L and ≤1 mg/L), the following interpretive breakpoints for disk diffusion susceptibility tests with 10-µg ceftibuten disks were calculated using regression line analysis: ≤19 mm for resistance and ≥27 mm for susceptiblity. Proposed inhibition zone diameters for the reference strain *Escherichia coli* ATCC 25922 are between 31 and 36 mm.

Keywords: Ceftibuten, regression analysis, disk diffusion, susceptibility test, MIC

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INTRODUCTION

Ceftibuten is an orally administered cephalosporin that is stable against plasmid-mediated β-lactamases [1,2], including extended-spectrum β-lactamases [3]. It exhibits antimicrobial activity against many Gram-negative and selected Gram-positive organisms. Clinical efficacy has been demonstrated in the treatment of upper and lower respiratory tract infections and complicated and uncomplicated urinary tract infections. Compared to older oral cephalosporin antibiotics, it has improved pharmacokinetic properties. Ceftibuten is well absorbed (75–90%) from the proximal gastrointestinal tract after oral administration [4,5]. Peak plasma levels (C_{max}) after single oral doses of 200 and 400 mg reached 9.9 and 17 mg/L, respectively [6]. Its half-life is 2.5 h in adults [4]. For several indications, ceftibuten can be given once a day. Minimal inhibitory concentration (MIC) breakpoints recommended by the DIN (German Institute for Standardization) [7] are ≥8 mg/L for resistance and ≤1 mg/L for susceptibility. This study aimed to establish appropriate interpretive criteria for susceptibility tests in ceftibuten by using the disk diffusion method according to DIN 58 940.

MATERIALS AND METHODS

A total of 275 bacterial strains of Gram-positive and Gram-negative bacteria, including 11 different species with 25 strains each (Table 1), were included in the study. They were epidemiologically unrelated recent clinical isolates from the Institute for Medical Microbiology in Leipzig, Germany. Ceftibuten was obtained from Essex Pharma GmbH (München, Germany; Lot. no. 8057). Iso-Sensitest media (Iso-Sensitest broth, Iso-Sensitest agar, Oxoid, Basingstoke, UK) were used for both microbroth dilution and disk diffusion tests.

For microbroth dilution assays, ceftibuten was dissolved in 0.1 M phosphate-buffered solution (pH 8.0) according to the manufacturer's recommendations (Shionogi and Co. Ltd, Iwate,

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Table 1 *In vitro* activity of ceftibuten against 275 bacterial strains

Species (25 strains each)	No. of isolates with MIC (μg/mL)												
	≤0.02	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	≥128
Acinetobacter baumanii	–	–	–	–	–	–	–	–	–	–	3	16	4	2
Citrobacter freundii	–	–	2	–	4	8	8	–	–	–	–	–	1	2
Enterobacter aerogenes	–	–	–	–	3	7	5	1	3	2	–	1	1	2
Enterobacter cloacae	–	–	–	1	1	2	8	2	–	–	–	–	–	11
Escherichia coli	–	–	3	5	8	5	–	–	1	1	2	–	–	–
Klebsiella oxytoca	–	11	5	3	5	1	–	–	–	–	–	–	–	–
Klebsiella pneumoniae	–	–	12	9	2	–	1	–	1	–	–	–	–	–
Morganella morganii	–	1	7	6	3	1	–	–	1	3	2	–	–	1
Proteus mirabilis	5	13	7	–	–	–	–	–	–	–	–	–	–	–
Proteus vulgaris	3	12	9	1	–	–	–	–	–	–	–	–	–	–
Serratia marcescens	–	–	–	4	9	3	1	3	3	2	–	–	–	–

Japan). Appropriate dilutions of this stock solution were made in Iso-Sensitest broth and dispensed into microdilution trays (Greiner, D-72636 Frickenhausen, Germany), which were stored until use at −80 °C.

Disk diffusion tests were performed according to DIN 58 940–3 [7]. Disks loaded with either 10 μg or 30 μg ceftibuten were obtained from Mast Diagnostics (Merseyside, UK; 10 μg, Lot No/Ch.-B 084390; 30 μg, Lot No/Ch.-B 081852) and stored at −20 °C. Zones of inhibition were measured with calipers after incubating for 18 ± 2 h at 36 ± 1 °C.

The correlations between zone diameters and MIC values were determined by regression analysis according to the method of least squares. In order to restrict the evaluation to the linear portion of the parabolic regression curve, the regression statistic was calculated using only isolates with MICs between 0.03 and 32 mg/L.

To establish reference values for ceftibuten disk tests with the reference strains recommended by DIN 58 940–3 [7], zone diameters were determined for both disk loads using disks from two different commercial manufacturers (Mast Diagnostics and Becton Dickinson Co., Cockeysville, MD, USA; 10-μg, Lot. 1005 704638; 30 μg, Lot. 1006 804527). For *Escherichia coli* ATCC 25922, a total of 40 readings for each disk load were performed (five separate agar plates for each disk load, respectively, were investigated on 4 successive days). Since all other strains recommended by the DIN for quality control purposes were resistant, 10 readings for each disk load were regarded as sufficient in these cases.

RESULTS

Table 1 shows the species-dependent distribution of ceftibuten MIC values. These values do not reflect the present epidemiological situation in Leipzig, since efforts were made to select strains representing an even distribution of MICs over the range of concentrations tested, in order to obtain a meaningful statistic correlation. Among the *Enterobacteriaceae,* resistant strains with MICs ≥ 8 mg/L were only found for *Enterobacter* spp., *Morganella morganii*, *Serratia marcescens*, *Citrobacter freundii* and *Esch. coli*. For individual strains of *Klebsiella pneumoniae,* MICs ranged up to 2 mg/L, being just above the susceptibility threshold. *Proteus* spp. were susceptible to 100% with a MIC_{90} of 0.06 mg/L. Altogether, median MICs for different species of the *Enterobacteriaceae* encompassed values from 0.03 to 2 mg/L. All strains of *Acinetobacter baumanii* were resistant to ceftibuten (MIC_{50} 32 μg/mL). Figures 1 and 2 show the test results as scattergrams comparing ceftibuten MICs with 10-μg and 30-μg ceftibuten disk zone diameters, respectively. Table 2 presents the statistical data of the regression analysis with 10-μg and 30-μg ceftibuten disks. For both disk loads, MICs and zone diameters corresponded to correlation coefficients of $r = -0.97$. However, there was a significantly greater number of very large inhibition

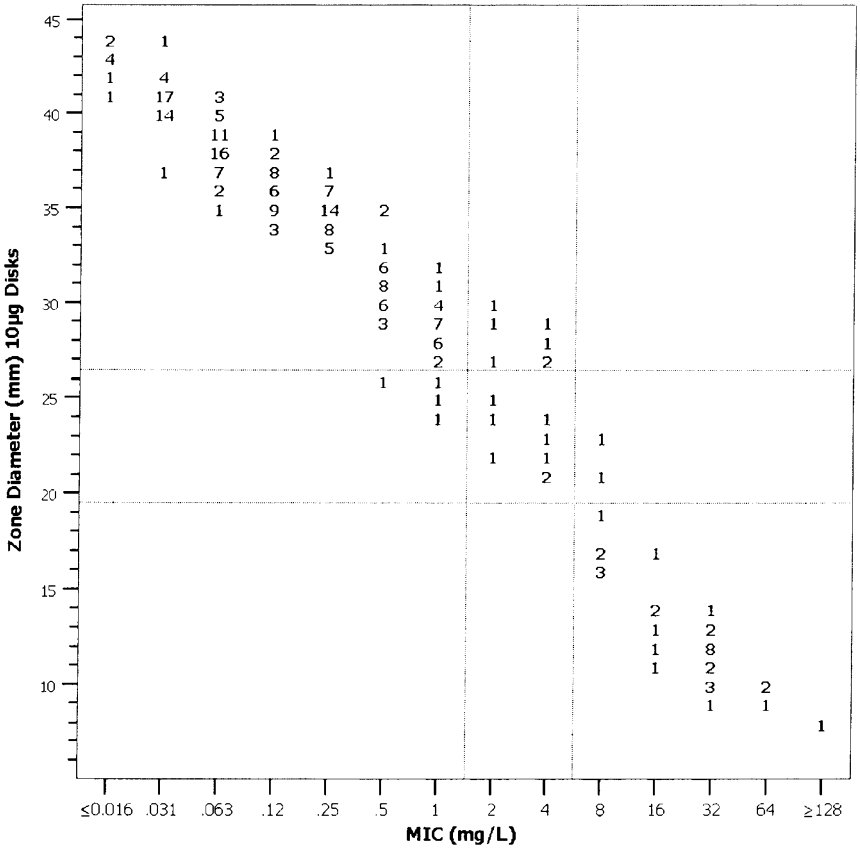

Figure1 Scattergram of ceftibuten broth microdilution MICs versus 10-µg disk zone diameters on Iso-Sensitest agar using the DIN method.

zones (17.3% vs. 8.9% zones >40 mm) produced by the 30-µg disks than by the 10-µg disks. Based on the MIC breakpoints recommended by DIN 58 940–4 [7] (≤ 1 mg/L, ≥ 8 mg/L), 10-µg disks and the calculated regression formula, the zone size criteria are as follows: resistant ≤ 19 mm, intermediate 20–26 mm and susceptible ≥ 27 mm.

For the 10-µg disks there were no very major discrepancies, no major discrepancies and 13 minor errors (5.1%). In spite of the very good correlation, *Serratia marcescens* produced noticeably larger than average inhibition zones, contributing significantly to the error rate.

Table 3 shows the summarized results of the susceptibility tests for the reference strain *Esch. coli* ATCC 25922. Clinically significant differences between disks produced by different manufacturers were not noted. As expected, *Pseudomonas aeruginosa* ATCC 27853, as well as the Gram-positive reference strains *Staphylococcus aureus* ATCC 29213, ATCC 25923 and *Ent. faecalis* ATCC 29212 recommended by DIN 58 940–3 [7]

Table 2 Statistical data of regression analysis[a] and interpretive criteria for ceftibuten disks

Disk potency	n[b]	r	Regression formula	Zone diameter (mm) Susceptiblity	Resistance
10-µg	243	−0.97	$x = -0.33\,y + 18.12$ $y = -2.84 \times + 53.10$	27	19
30-µg	243	−0.97	$x = 0.37\,y + 20.10$ $y = -2.54 \times + 53.33$	30	22

[a] Regression analysis for MIC range 0.03–32 mg/L; [b] without extreme values; x = MIC as the $\log_2 + 9$; y = Zone diameter (mm).

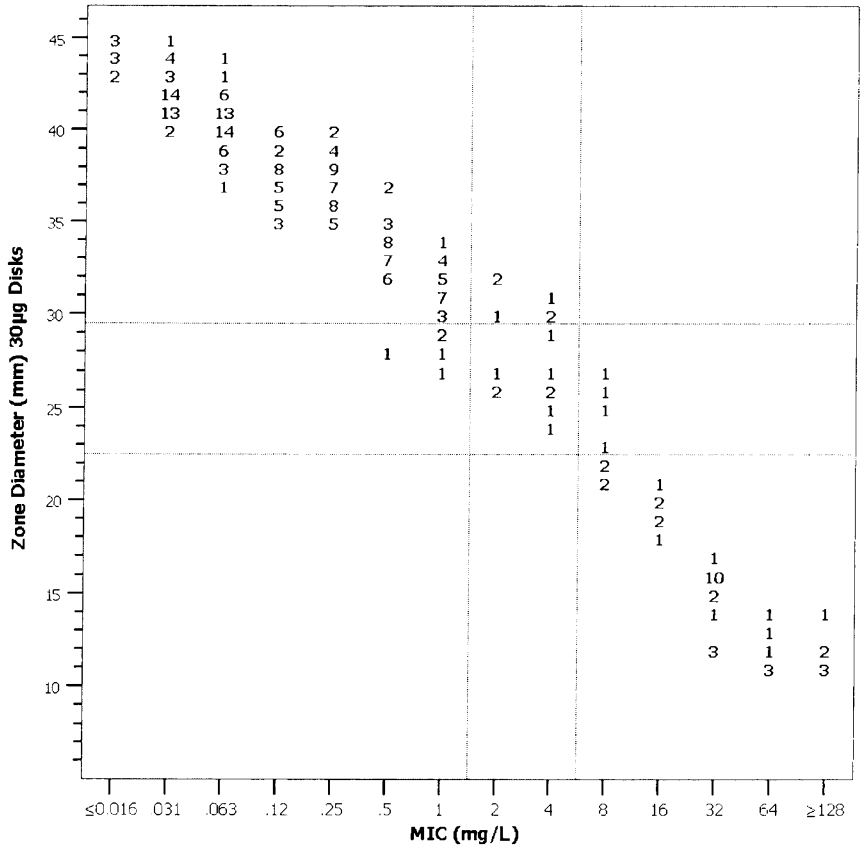

Figure 2 Scattergram of ceftibuten broth microdilution MICs versus 30-µg disk zone diameters on Iso-Sensitest agar using the DIN method.

Table 3 Evaluation of susceptibility tests with ceftibuten disks for the reference strain *Esch. coli* ATCC 25922

Organism	Disk potency (µg)	No. of replicate tests	Statistical data of zone diameters Median (mm)	MIC SD	Median (mg/L)
Esch. coli ATCC 25922	10	40	34	0.65	0.25
	30	40	37	0.72	

for quality control purposes, are resistant to ceftibuten and consequently were not useful for quality control purposes.

DISCUSSION

A problem in the interpretation of bacterial susceptibility to antimicrobial agents is the difference between national standards by which these susceptibilities are determined, leading to different interpretations for the same antibiotic in different countries. According to the NCCLS, where the determination of MIC breakpoints for ceftibuten is mainly based on peak serum concentrations, the recommendations are ≤ 8 mg/L for susceptibility and ≥ 32 mg/L for resistance[8]. The respective values from the DIN are ≤ 1 µg/mL and ≥ 8 µg/mL, since calculations are based to a greater extent on mean serum and tissue concentrations [9].

For susceptibility tests of cephalosporins with the agar diffusion method, disks with 30 µg potency are common internationally. However, some of the recently developed cephem antibiotics, including ceftibuten, exhibit an increased antibacterial activity. Along with the comparably light inoculum of the DIN method and the use of Iso-Sensitest media, the high potency disks led to a great number of very large inhibition zones. Correct reading of zone diameters of other antimicrobial substances can be seriously disturbed when several disks with large inhibition zones are used on one agar plate. For ceftibuten,

the correlation between MIC and zone diameter is very good for both disk loads; however, the number of large inhibition zones is significantly lower for the 10-μg disks, rendering this potency favorable for susceptibility testing. Disks with an even lower potency are currently not readily available commercially and therefore are not recommended here.

For quality control purposes of ceftibuten susceptibility tests, only *Esch. coli* ATCC 25922 is well suited as a reference strain, since the other strains recommended by DIN 58 940 result in too small or no inhibition zone diameters. MICs for *Esch. coli* ATCC 25922 should range between 0.125 and 0.5 mg/L. The MIC values obtained in our studies are in good agreement with the values established previously by Jones and Barry [1]. We concluded from the replicate disk diffusion tests performed with the *Esch. coli* reference strain that the corresponding inhibition zone diameters achieved with DIN 58 940 should be in the range of 34 ± 3 mm.

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